THE ELECTRON MICROSCOPY OF F-ACTIN

by

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Through its ability to portray particles of macromolecular dimensions, the electron microscope is affording a new approach to problems of the fine structure of muscle. Of the two possible methods of attack, one deals with the fine structure of intact muscle fibres, the other with the macromolecular particles of proteins that can be extracted from muscle. We have been employing both methods in combination with one another and with developing chemical studies. This paper records observations on the fine structure of actin as one of the proteins isolated from muscle.

Evidence for the existence of actin was first obtained by BANGA AND SZENT-GYÖRGYI[1], who found that the solution prepared by extracting muscle for 24 hours with Edsall's salt solution had an extraordinarily high viscosity. This actin, as isolated by methods worked out by STRAUB[2], has exhibited properties that have made it the classical example of a substance showing a globular-to-fibrous (G-F) molecular transformation. Isolated from muscle dried with acetone, it is a protein of low viscosity which when made 0.1 M with respect to KCl and allowed to stand becomes more viscous and ultimately turns into a thick thyxotropic gel. Everything that has since been learned about this change has confirmed the interpretation of SZENT-GYÖRGYI AND STRAUB that actin does indeed occur in two forms, a globular and a fibrous modification.

Two electron microscopic investigations, by JAKUS AND HALL[3] and by ASTBURY, PERRY, REED, AND SPARK[4], have revealed the particles of F-actin as long, structureless threads; beyond indicating that their molecules must be small, all results on G-actin have been equivocal. In this article we are concerned with a possible fine structure in the F-actin threads, with the details of how they arise from globular actin and with the relation between them and particles of similar dimensions that can be seen in muscle fibrils.

In all the present experiments globular actin was extracted from excised rabbit muscle according to GUBA's modification of the method of STRAUB. Actin threads were made by adding 0.1 M KCl to a solution of this actin and waiting for the G-F transformation to take place at room temperature. In a first set of experiments drops of this transformed actin were put on collodion covered grids, the excess was withdrawn and salt was removed by washing with distilled water; these cleaned preparations were then shadowed with chromium or palladium and examined in an RCA-type EMU electron microscope. A concentrated actin solution gave the kind of network described by ASTBURY, PERRY, REED, AND SPARK. Where fine threads could be observed among its

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Fig. 1. Separate fibrils of F-actin resting on a collodion substrate. Magnification = 29000 ×

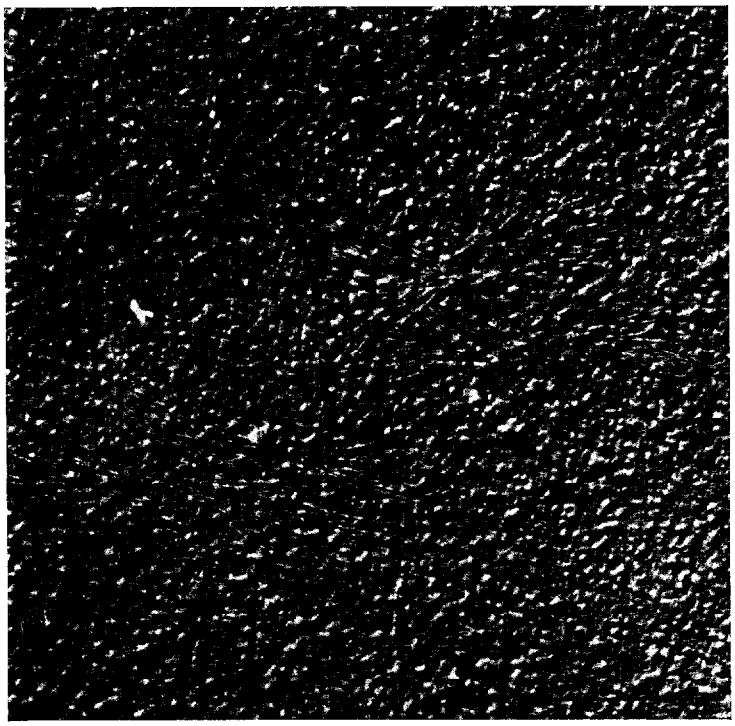

Fig. 2. Sheaves are fibres of F-actin beginning to form in a thick mass of G-actin. Magnification = 25000 ×

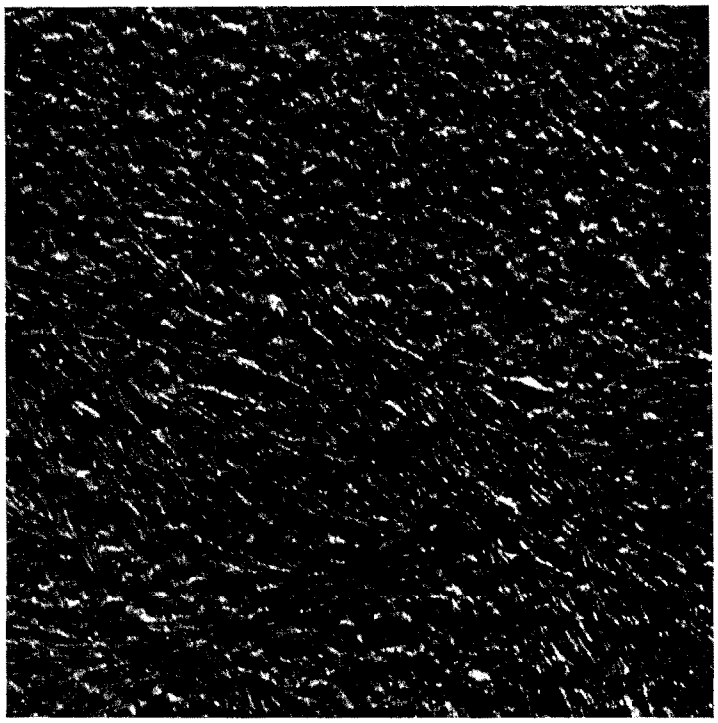

Fig. 3. A preparation similar to that of Fig. 2, seen at higher magnification (43000 ×)

Fig. 4. Single fibres of F-actin extending from a mass of F-actin resulting from the *in situ* conversion of G-actin. Magnification = 45000 ×

Fig. 5. Groups of F-actin fibres formed *in situ* by conversion from G-actin. Magnification = 38000 ×

bundles, no detail was seen within them. A more dilute actin solution resulted in preparations (Fig. 1) containing very long fine threads with the dimensions of those pictured by JAKUS AND HALL. These individual threads were so thin that roughness of the collodion substrate interfered with their portrayal and confused any attempts to observe their fine structure.

In the effort to avoid this difficulty another set of experiments was made in which the transformed actin was deposited on a microscope slide, washed, shadowed with palladium and removed as a "pseudo-replica" with the help of the usual reinforcing collodion film. Unfortunately, these "pseudo-replicas" were not more successful than direct deposits on collodion in revealing fine structure within actin threads.

In a third and more rewarding group of experiments, the G-F transformation was carried out directly on the glass slide which could then be "replicated" without any disturbance of the fibrous structure as formed. To do this, freshly extracted globular

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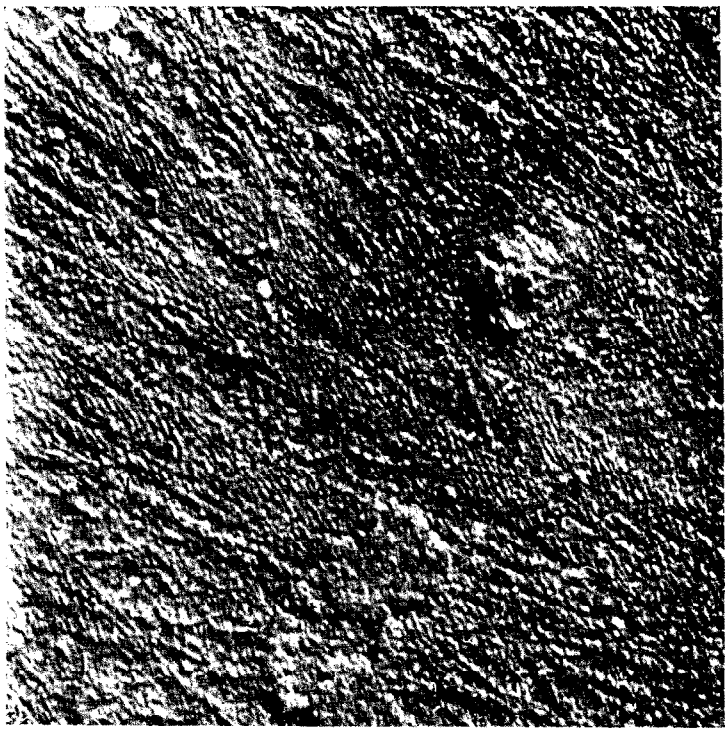

Fig. 6. Another field in an F-actin preparation formed *in situ* from G-actin. Magnification = 50000 ×

actin containing a minimal amount of KCl instead of the previous 0.1 M quantity was dropped onto the slide and allowed to evaporate. At a certain point in this evaporation, the small amount of salt present reached the necessary concentration and fibrous actin formed on the slide. Interfering KCl crystals have rarely been found in such preparations and the threads have not been disturbed after their formation by the pipetting and stirring involved in handling preformed fibres.

Various stages of fibre formation can be observed in preparations made in this way. An initial step in the transformation to fibres is illustrated in Fig. 2 where threads can be seen developing in sheaves from a few points of origin. The single threads in these bundles appear against a background whose unevenness is due to its being a thick mass of untransformed globular actin. Fig. 3 shows at a higher magnification a slightly more advanced state in the transformation. Detail cannot be seen within the fibres of either photograph, presumably because the preparations are very thick.

There is less interference from globular actin as the transformation proceeds; if less concentrated globular actin solutions are used, preparations ultimately will be obtained that show only the fibrous form (Fig. 4). The fibres thus prepared have a uniform thickness of about 100 Å; they are very long, do not branch and do not split into finer threads at their ends. Most isolated threads seem devoid of internal structure, but in places at the lower right of this photograph there are indications of their being built of units *ca* 300 Å long whose long axes do not necessarily coincide with the fibre axes.

A conspicuous cross striation appears when the transformation takes place under

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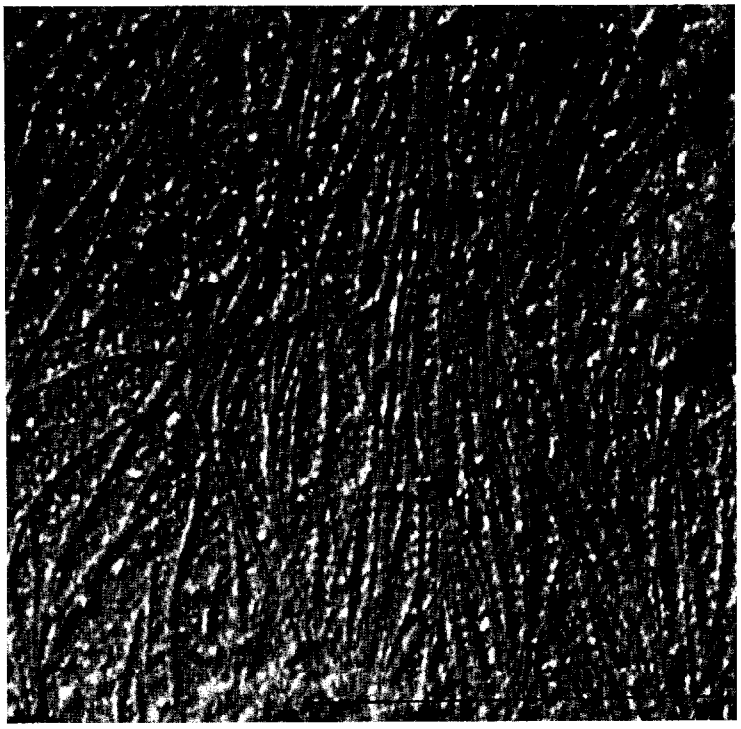

Fig. 7. A part of the preparation of Fig. 5 seen at a higher magnification (55000 ×)

Fig. 8. Another preparation of F-actin formed *in situ*. Magnification = 41000 ×

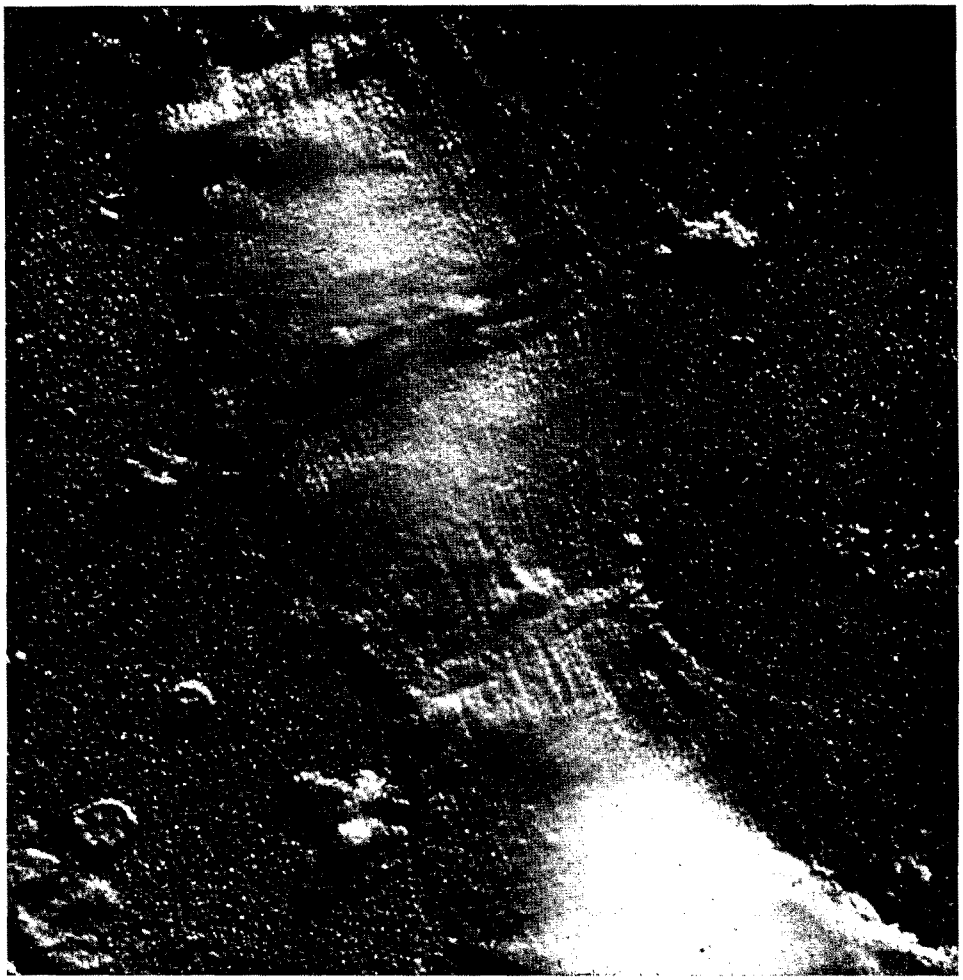

Fig. 9. Part of a minute, intact fibril of rabbit muscle showing its macromolecular fibrillar structure. Magnification = 37000 ×

conditions such that the resulting fibres are in contact to form parallel closely packed aggregates. The cross striation is seen when no more than two threads are in contact but is more evident in broader bundles whose cross segmentation and lateral association are often so orderly as to give the impression of a regular net (Fig. 5). Frequently the cross bands are more conspicuous than the original fibres (Fig. 6).

The single actin filaments seemingly are lengthwise associations of ellipsoidal rodlets *ca* 300 Å long and *ca* 100 Å wide. The cross striation is the result of a regular side-by-side association of these particles (Figs 5 and 7). In many photographs this cross banding stands at an angle of 90° to the original fibre axis but in most pictures its angle is smaller, down to 20°. In many fields all fibres show about the same angle. Fig. 6 is a further good example of this two-dimensional regularity in arrangement.

Many fibres, such as those shown in Figs 7 and 8, give the impression that the common axis of the parallel particles composing them may be inclined to the fibre axis.

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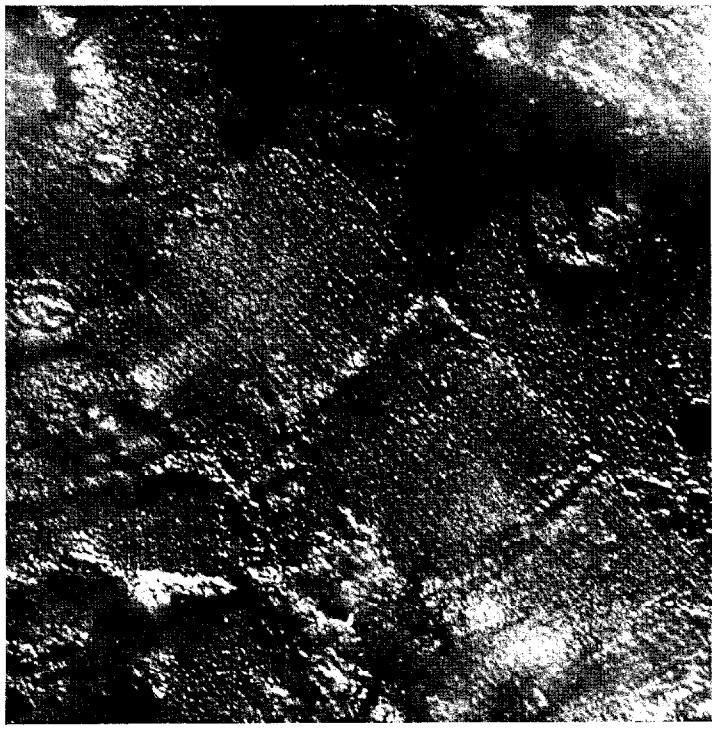

Fig. 10. A portion of another intact muscle fibril from the rabbit. Magnification = 29000 ×

Fig. 11. Elementary fibrils obtained by the mechanical disintegration of muscle fibres such as those of the two preceding figures. Magnification = 30000 ×

The lengthwise association of the particles of a fibre would then be not strictly end-to-end but intermediate between end-to-end and side-by-side.

It is important to compare these results with what is known of the size of the actin molecule. An ellipsoidal protein particle having the dimensions of the segment seen in these electron micrographs (*ca* 300 Å by *ca* 100 Å) would have a molecular weight of *ca* a million and a half. This is much bigger than the known weight of the molecule of globular actin which according to both STRAUB[6] AND SNELLMAN, TENOW AND ERDÖS[7] is *ca* 70000. Results are understandable, however, if the real G-F transformation in actin is a polymerization of 70000 weight molecules to form the ellipsoidal elements seen in the electron micrographs and if these polymerized units then invariably associate together to form fibres and bundles.

The observations of fine detail in F-actin deposits raise questions as to their bearing on the structure of muscle. Intact fibrils small enough to reveal their macromolecular constitution appear as in Figs 9 and 10. Evidently they are in large measure bundles of filaments that have the overall dimensions of the F-actin threads. If bits of muscle are disintegrated in a Waring blendor these filaments are liberated. After washing they appear as in Fig. 11. The similarity between them and freshly formed threads of F-actin is made clear by comparing this figure with Fig. 4. The threads in the intact muscle of Figs 9 and 10 are for the most part independent of one another, but small regions can be found which exhibit an approach to the two-dimensional order seen in the actin photographs of this paper. We are examining further this implication that the filaments in intact muscle may be related to the corresponding particles of F-actin.

SUMMARY

Electron micrographs have been made of deposits of F-actin polymerized *in situ* from the globulin form. Aggregates of fibers obtained in this way show a new cross segmentation and lateral association which indicates a fine structure within the single fibers.

RÉSUMÉ

On a fait au microscope électronique des photographies de sédiments de F-actine polymérisée *in situ* à partir de la forme globuline. Les agrégats de fibres obtenus de cette manière montrent une nouvelle segmentation croisée et une association latérale, qui indiquent une structure fine à l'intérieur des fibres simples.

ZUSAMMENFASSUNG

Elektronen-Mikrogramme wurden aufgenommen von *in situ* aus der Globulinform polymerisierten Ablagerungen von F-Aktin. Fiber-Aggregate, die auf diese Weise erhalten werden, lassen eine neuartige gekreuzte Unterteilung und eine seitliche Vereinigung sehen, welche eine Feinstruktur innerhalb der einzelnen Fibern zeigen.

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